Technical Note

Increased Enzyme Concentration for the GeneChip WT cDNA Synthesis and Amplification Kit

Abstract

Affymetrix has enhanced the efficiency of the amplification during the IVT reaction of the GeneChip® WT cDNA Synthesis and Amplification Kit by increasing the concentration of T7 RNA Polymerase in the IVT Enzyme Mix.

Benefits of the change include

- **significantly greater cRNA yields with no change to current protocol**
- **same high-quality results**
- **more samples that will meet yield requirements**

To compare the performance of the increased enzyme concentration to the former, target was generated from MAQC A and B total RNA samples at three external sites. Average cRNA yield for the new enzyme concentration increased by 96 percent (MAQC A) and 82 percent (MAQC B) relative to the previous concentration. When hybridized to the GeneChip® Human Exon 1.0 ST Array, average probe set signals from the increased enzyme concentration showed very high levels of correlation ($R^2 > 0.99$) to the former enzyme concentration.

This technical note presents results from three external comparison studies which demonstrate that the new, increased enzyme concentration dramatically increases cRNA yield without significantly impacting array data.

Introduction

The GeneChip® WT cDNA Synthesis and Amplification Kit is designed to generate target for GeneChip® Gene 1.0 ST and Exon 1.0 ST Arrays. The kit employs a random priming strategy during reverse transcription to generate cDNA from the entire length of each RNA transcript. After synthesizing the second strand, linear amplification of the message is achieved using in vitro transcription (IVT). The resulting cRNA is converted back into single-stranded sense strand cDNA, which is then fragmented and labeled by utilizing the GeneChip® WT Terminal Labeling Kit before hybridization to the array.

Affymetrix has developed a new IVT Enzyme Mix that enhances the efficiency of the amplification during the IVT reaction. The only change is an increase in the concentration of T7 RNA Polymerase. The new enzyme concentration has been rigorously tested and validated internally and at several external sites. Array data generated from the new enzyme concentration is nearly indistinguishable from the previous concentration. In this study, we present results from external beta testing at three independent sites where the new enzyme concentration was directly compared to the previous concentration using multiple total RNA samples.

Methods

The experiment was performed at three external sites, all of which were experienced in performing the whole-transcript (WT) assay. At each site, WT target was generated from the MAQC A (Stratagene Universal Human Reference RNA; catalog No. 740000) and MAQC B (Ambion Brain Reference RNA; catalog No. 6050) using the GeneChip® WT cDNA Synthesis and Amplification Kit, starting with 1 µg of total RNA. For each sample, the levels of ribosomal RNA (rRNA) were reduced using the Invitrogen RiboMinus Kit (catalog No. K155001).

Three technical replicates from each RNA sample were generated from the two enzyme concentrations for a total of 12 samples (see Table 1). For the higher concentration samples, the

Table 1: Samples processed at each site.

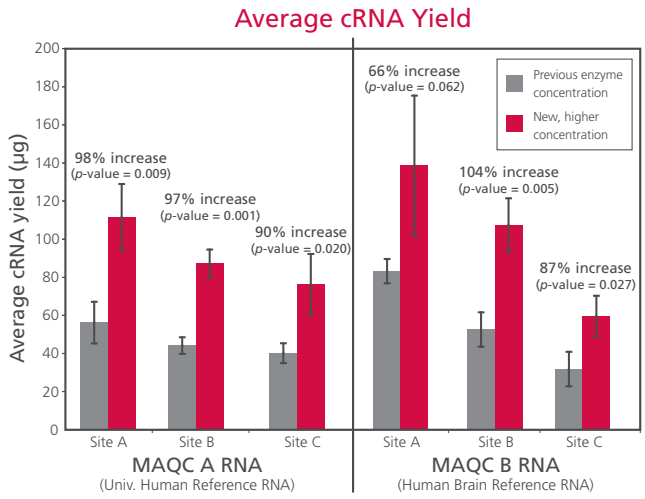
Sample #	cDNA Amplification Kit enzyme concentration	Total RNA	Replicate #
1	Previous concentration	MAQC A	1
2	Previous concentration	MAQC A	2
3	Previous concentration	MAQC A	3
4	Previous concentration	MAQC B	1
5	Previous concentration	MAQC B	2
6	Previous concentration	MAQC B	3
7	New, higher concentration	MAQC A	1
8	New, higher concentration	MAQC A	2
9	New, higher concentration	MAQC A	3
10	New, higher concentration	MAQC B	1
11	New, higher concentration	MAQC B	2
12	New, higher concentration	MAQC B	3

IVT Enzyme Mix from the GeneChip® WT cDNA Synthesis and Amplification Kit was replaced with the higher concentration enzyme in the IVT step. Labeled target was hybridized to the GeneChip® Human Exon 1.0 ST Array. After scanning, the resulting CEL files were sketch-quantile normalized and the core gene-level probe set values were summarized using RMA in Expression Console™ Software.

Results

Average cRNA yield was calculated at the three sites for each of the total RNA samples. The results are displayed in Figure 1. Gray bars represent the previous enzyme concentration and red bars represent the new concentration, with MAQC A samples on the left and MAQC B samples on the right.
In each case, the average cRNA yield was higher when the increased enzyme concentration was used. Error bars indicate one standard deviation (n = 3). For each site, a t-test was used to determine the statistical significance of the yield difference. *P*-values for the difference between previous and new enzyme concentrations are displayed above the bars for each site. As shown in Table 2, when the data is compiled from all three sites, the MAQC A sample shows a 95.6 percent increase in cRNA yield with the increased enzyme concentration (t-test *p*-value of 1.63×10^{-5}) and the MAQC B sample shows an 82.3 percent increase (t-test *p*-value of 8.98×10^{-3}).

Figure 1: Average cRNA yield at each site.



Core gene-level data was generated using the RMA algorithm in Expression Console™ Software, and averages were calculated across the three replicates at each of the three sites. Average gene-level signals from the previous enzyme concentration were directly compared to the increased concentration to determine if the change produced any bias in the array signal.

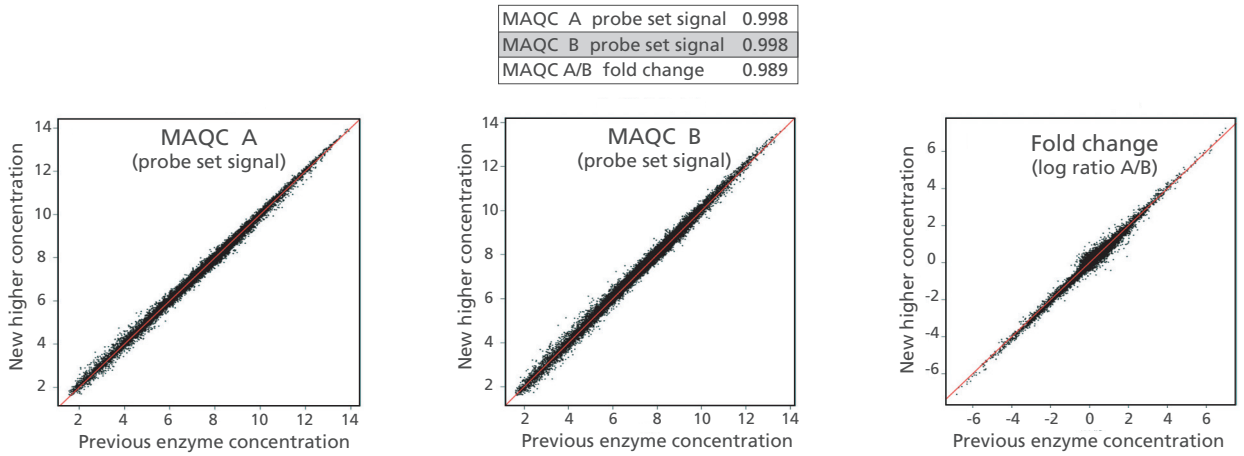
Scatter plots for the MAQC A and B samples are shown in Figure 2. The Pearson correlation coefficients (R^2) for both are greater than 0.99 and no significant bias in array data was detected. The average fold change [log (MAQC A / MAQC B)] was also calculated for each gene-level probe set. Again, the replicates from each of the three sites from the previous enzyme concentration were compared to new enzyme concentration (Figure 2). The Pearson correlation coefficient was 0.989, showing highly similar performance of the two formulations.

Using the gene-level data for each of the samples at all three sites, a multi-factor ANOVA was carried out to explore the contributions of each of the parameters. The sources of variation are graphically represented in Figure 3. As expected, the RNA sample type was by far the largest contribution to

Table 2: Average cRNA yield for each sample.

Sample	Enzyme concentration	Replicate #	Yield site A	Yield site B	Yield site C	Average cRNA yield (µg)	Std. dev.	Increase	T-test *p*-value
MAQC A	Previous	1	50.0	44.7	38.1	46.9	9.7	95.6%	1.63×10^{-5}
		2	50.0	39.6	46.1				
		3	68.9	48.3	36.3				
	New	1	124.2	91.7	94.4	91.7	19.9		
		2	91.8	78.6	70.0				
		3	118.8	91.2	64.6				
MAQC B	Previous	1	78.3	44.2	41.4	55.9	23.5	82.3%	8.98×10^{-3}
		2	81.0	51.5	30.9				
		3	90.5	62.1	23.4				
	New	1	97.2	92.4	64.6	101.9	40.0		
		2	151.2	110.5	66.9				
		3	167.4	119.8	47.3				

Figure 2: Average gene-level probe set signal and fold change correlation (Pearson correlation coefficient).



variation in the data set. Variation from the enzyme concentration was negligible relative to real biological differences in the data.

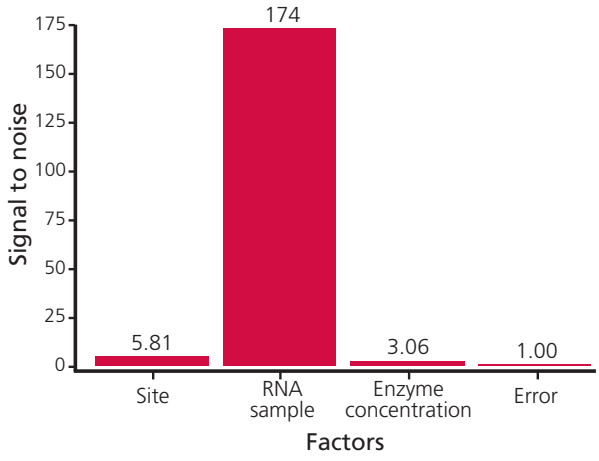
Conclusion

As demonstrated by the data presented in this technical note, the incorporation of an increased enzyme concentration in the IVT Enzyme Mix from the GeneChip® WT cDNA Synthesis and Amplification Kit resulted in a dramatic increase in cRNA yield with no significant impact on array results.

References

GeneChip® Whole Transcript (WT) Sense Target Labeling Assay Manual (version 4)
http://www.affymetrix.com/Auth/support/downloads/manuals/wt_sensetarget_label_manual.pdf

Figure 3: ANOVA sources of variation.



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